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(54) Title: GALANIN (57) Abstract This invention relates to galanin and its uses. In particular, the invention provides a knockout mouse which lacks a functional galanin gene. The mouse may be used to investigate the effects of galanin. It has also been unexpectedly discovered that galanin antagonists may be used in the management of pain, particularly painful neuropathies, the suppression of pain, in the suppression of lactation, in the treatment of prolactinoma and in anaesthesia. Additionally, the use of galanin agonists may be used in the treatment of Alzheimer's disease, in improving memory and cognition and in the treatment of nerve damage, such as the promotion of nerve regeneration.		

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GALANIN

This invention relates to galanin, including analogues thereof and its uses.

Galanin is a 29 amino acid neuropeptide which was first isolated from porcine intestine in 1983. Subsequently, the cDNA for galanin was cloned from a rat anterior pituitary library in 1987. Nucleotide and amino-acid sequence analysis suggests that galanin is unrelated to any of the other known families of regulatory peptides, and remains the only member of its family. The N-terminal portion of galanin is highly conserved between species, there being variation in the C-terminal portion.

Galanin has a widespread distribution in the peripheral and central nervous systems, gut and pancreas. It is found in highest levels in the median eminence of hypothalamus and in the pituitary

WO92/12997 (General Hospital Corporation), published in 1992, discloses the sequence of human galanin. There is a discussion of studies by other workers involving the administration of rat galanin or its N-terminal fragments to augment the effect of morphine and this patent application suggests that galanin can be expected to exhibit analgesic effects such that it may be administered alone or in combination with other analgesics. The application claims the use of galanin or its analogues in the treatment of pain and the use of galanin antagonists in the treatment of certain other conditions.

WO92/20709 (Astra AB) discloses a number of putative galanin antagonists. The antagonists which are described are all based on the first 12 amino acids of galanin followed by partial sequences of other peptides i.e. chimeric peptides. Some may be agonists, some antagonists and some may be both depending on the receptor subtype. The application discloses that the antagonists may be useful for treatment of insulin-, growth hormone-, acetyl choline-, dopamine-, Substance P-, Somatostatin-, and noradrenaline-related conditions including endocrinology, food intake, neurology and psychiatry, Alzheimer's type dementia, analgesia, intestinal disease. The application discloses the results of studies using some of the antagonists described therein on various

effects such as galanin inhibition of glucose stimulated insulin release; galanin induced inhibition of scopolamine induced ACh hippocampal release; galanin induced facilitation of the flexor reflex; the displacement of bound iodinated galanin in membrane binding studies. There is a suggestion in the application that the antagonists may be indicated for analgesia but there is no disclosure in the application of results to this effect.

Approximately 2-4% of the Western population suffer from diabetes mellitus and, of those people, 10-15% suffer from chronic pain and numbness in their extremities-termed "painful neuropathy". Present techniques for management of painful neuropathy are inadequate.

Alzheimer's disease is a major cause of morbidity worldwide the disease being characterised by loss of memory and personality changes. At an anatomical level there is a major decrease in the number of cholinergic nerves in the hippocampus, which is the main area of the brain thought to process and store memories. Previous work has shown that galanin is also expressed in these hippocampal nerves and the levels of galanin are two fold elevated in the brains of patients with Alzheimer's disease.

The present invention relates to the generation of a mouse with targeted disruption of the galanin gene; experiments using the mouse, and the implication of the results of those experiments for the treatment of disease. In particular, the invention relates to the generation of a mutant mouse carrying a loss-of-function germ-line mutation of the galanin locus. The inactivating mutation has been introduced into the mouse genome utilising targeted mutagenesis in embryonic stem cells by homologous recombination. The mutation, when bred to homozygosity on the inbred 129sv background, affects feeding behaviour, lactation and pain sensitivity. The mutation may also affect memory and behaviour, sexual reproduction and fertility and insulin secretion with resultant changes in circulating blood glucose levels.

According to first aspect of the invention there is provided the use of a galanin agonist in the preparation of a medicament for the treatment of nerve damage.

According to a second aspect of the invention there is provided a method of healing, preferably repairing, nerve damage in a subject comprising administering to the subject a galanin agonist.

According to another aspect of the invention there is provided a method of treatment of Alzheimer's disease and related diseases and conditions, the method comprising administering a galanin agonist to a subject.

In a further aspect of the invention, there is provided a method of improving memory, enhancing memory and improving cognitive function, comprising administering a galanin agonist to a subject. Advantageously, such treatment may be used in the treatment of restoring memory after injury or trauma.

According to a further aspect of the invention there is provided the use of a galanin antagonist in the preparation of a medicament for the suppression of lactation and also a method of suppressing lactation in a mammal, the method comprising administering a galanin antagonist to that mammal.

According to another aspect of the invention there is provided a composition comprising a galanin antagonist for the treatment of prolactinoma in a mammal and also the use of a galanin antagonist in the preparation of a medicament for the treatment of prolactinoma and a method of treating prolactinoma in a mammal suffering from prolactinoma, the method comprising administering a galanin antagonist to that mammal.

The invention further provides galanin agonists suitable for use in the treatment of Alzheimer's disease, related diseases and conditions and in the improvement of memory and cognitive function. Also, the invention provides the use of a galanin agonist in the preparation of a medicament for the treatment of Alzheimer's disease and related diseases and conditions, and in enhancing memory and cognitive function.

According to a further aspect of the invention there is provided an analgesic composition comprising a galanin antagonist and, in addition, the use of a galanin antagonist in the preparation of a medicament for the treatment of pain.

According to a further aspect of the invention there is provided a method of suppressing pain in a mammal, the method comprising administering a galanin antagonist to that mammal and, in addition, the use of a galanin antagonist in the preparation of a medicament for the treatment of painful neuropathy.

According to a further aspect of the invention there is provided an appetite suppressant composition comprising a galanin antagonist and, in addition, the use of a galanin antagonist in the preparation of a medicament for the suppression of appetite. This aspect of the invention also provides a method of suppressing appetite in a mammal, the method comprising administering a galanin antagonist to that mammal.

According to a further aspect of the invention there is provided an anaesthetic composition comprising a galanin antagonist and, in addition, the use of a galanin antagonist in the preparation of an anaesthetic composition. This aspect of the invention also provides a method of anaesthetising a mammal, the method comprising administering a galanin antagonist to that mammal.

According to a further aspect of the invention there is provided a mammal, preferably a rodent, which lacks a functional galanin gene. The term "galanin" embraces all known galanins including, for example, human, rat, murine and porcine galanin and also analogues of galanin having the biological activity of galanin. The galanin gene may have been inactivated by at least partial deletion of the galanin gene sequence between the Bam HI and Bgl2 restriction sites indicated by asterisks in the accompanying Fig. 3. Where the mammal is a rodent, it is preferably a mouse. Other mammals such as sheep and rats are contemplated.

According to another aspect of the invention there is provided tissue, cells and cell lines derived from the mammal in accordance with the first aspect of the invention. Preferably,

the tissue, cells or cell lines include cells from pancreas, pituitary, cortex, dorsal root ganglia, or are derived from such cells.

The mammal or tissue, cells and cell lines of the invention may be used in an assay to study one or more biological effects of galanin. The biological effect may be selected from, for example, prolactin secretion, appetite, memory, behaviour, pain, autotomy following axotomy, growth or the repair of nerve damage.

Embodiments of the invention will now be described, by way of example only, with reference to the accompanying drawings Figures 1 to 16 in which:

Fig. 1 illustrates the genomic structure of mouse galanin;

Fig. 2 illustrates the targeting vector used in producing the rodent of the invention;

Fig. 3 illustrates the specific recombination event in the production of the rodent in accordance with the invention;

Fig. 4 illustrates the genotype of the progeny determined using Southern blotting and by PCR demonstrating identical results from the same litter derived from a mating of two heterozygote animals;

Fig. 5 illustrates the weight gain and final body weights of wild-type and mutant animals over the first 8 weeks of life;

Fig. 6 illustrates results of experiments on behavioural responses of intact adult mice to thermal and mechanical stimulation;

Fig. 7 illustrates the effect of galanin inactivation on autotomy behaviour after sciatic nerve section;

Fig. 8 illustrates the effect of galanin inactivation on short term regeneration of sensory neurons;

Fig. 9 illustrates the effect of galanin inactivation on long term regeneration of sensory neurons;

Fig 10 illustrates expression of an exon 6-specific riboprobe to study the distribution of galaninergic neurons in the brain and dorsal root ganglion of wildtype and mutant mice;

Fig. 11 illustrates the effect of galanin inactivation on anterior pituitary prolactin content;

Fig. 12 illustrates the effect of galanin inactivation on anterior pituitary thyroid stimulating hormone content;

Fig. 13 illustrates the effect of galanin inactivation on anterior pituitary growth hormone content;

Fig. 14 illustrates the effect of galanin inactivation on anterior pituitary luteinizing hormone content;

Fig 15 illustrates the effects of galanin inactivation on the generation of long term potentiation in the stratum radiatum area of the hippocampus; and

Fig 16 illustrates the effects of galanin inactivation on the generation of long term potentiation in the stratum oriens area of the hippocampus.

To generate a mouse knockout, that is the introduction into the mouse genome of either a loss- or gain-of-function mutation of a specific gene locus (according to the procedure described in Kuehn, M. R. *et al* Nature. 1987; 326: 295-8; Thomas, K. R. and Capecchi, M. R. Nature. 1986; 324: 34-8) , entails a number of steps:- (1) the cloning of the mouse genomic locus of interest; (2) the construction of a targeting vector such that the

locus/gene of interest is modified to inactivate or alter its structure and function in some way; (3) introduction of the targeting vector into an embryonic stem cell library and selection and identification of single cell clones in whom the appropriate correct targeting event has taken place and in whom the normal chromosomal number is unchanged; and (4) introduction of such clones into 3.5 day old blastocysts and the resulting chimeric mice mated to wild types of the opposite sex. The resulting offspring demonstrated to carry the mutation are thus heterozygotes and, by appropriate mating, homozygotes for the introduced mutation are bred.

As a first step the murine *galanin* gene was cloned. A mouse genomic library (Ehrich, E. *et al* Gene. 1987; 57: 229-37) was screened using the full length rat *galanin* cDNA as a probe under high stringency. Two cosmid clones were identified spanning 60Kb around the galanin locus. Using 5' and 3' probes from the rat cDNA a 14 Kb region of DNA containing the entire gene was subcloned and partially sequenced. From the genomic sequence, primers were designed complementary to untranslated exonic regions of the gene. A 630bp fragment was generated by RT-PCR (Kit supplied by INVITROGEN BV, The Netherlands) using adult female whole brain as a source of mRNA. Subsequent sequencing of this fragment demonstrated that mouse and rat galanin are 100% identical at the protein level and 94.8% at the nucleotide level. The genomic structure of the mouse gene (Fig. 1) is identical to that of the rat gene. The gene spans 4.8Kb and consists of six exons. The translation start site (AUG) starts at the first base of exon two, the coding region for galanin extends across exons three and four with the stop codon (UGA) in the middle of exon six.

Using the 14Kb subclone described above, a positive/negative selection targeting vector was constructed (Fig. 2). The mutation introduced removes the first five exons containing the entire coding region of the galanin peptide (Fig. 3).

In Fig. 3: A and B are the sites of the external probes used to screen the ES cells for the appropriate integration of the construct

Neo = neomycin resistance gene

HSV-TK= herpes simplex virus thymidine kinase gene

B = BamHI

E=EcoRI

X=XhoI

Bg=BglII

In particular, the targeting vector removes a 3.2Kb stretch of DNA and thus removes the first 5 exons of the galanin gene. The exact sites flanking the stretch of DNA removed are 5' - the Bam HI site 10bp downstream from the transcriptional start site and the 3' site is the BglII site in the middle of intron 5. These sites are indicated with asterisks in Fig. 3. Other sites that could be used are the same 5' site and a differing 3' XhoI site in intron 4 which would remove only 2.9Kb of DNA and thus remove only first 4 exons.

This vector was linearised and electroporated into the E14 embryonic stem-cell (ES) line (Hooper, M. *et al* Nature. 1987; **326**: 292-5). Restriction mapping of the wildtype locus with BglII generates a 9.3Kb fragment when probed with a 5' external probe (marked A, Fig 3), whilst the correctly targeted locus generates a 4.4 Kb fragment. In total, 9 clones were identified in which one allele of the galanin gene was correctly targeted by homologous recombination among 209 double resistant colonies yielding a targeting frequency of 4.3%. These nine clones were karyotyped, confirming euploidy, and injected into 3.5 day old blastocysts from C57BL/6 mice. Germ line transmission of the disrupted galanin locus was obtained from three separate ES cell clones. Genotype of the progeny was determined using Southern blotting and by PCR (Fig 4 demonstrates identical results obtained by Southern blotting and PCR screening on the same litter derived from a mating of two heterozygotes). The mutation has been bred to homozygosity on the in-bred 129sv strain and all data presented is from mice on this background.

1. Results of genotype analysis of live births are in the expected ratio predicted by Mendelian genetics and the sex ratio is 1:1. Galanin levels were measured by

radioimmunoassay and immunocytochemistry in areas previously demonstrated to express galanin at high levels and include brain, pituitary, spinal cord, dorsal root ganglion, stomach, small intestine and uterus. Galanin levels in heterozygotes for the deletion were 50% of wild type controls whilst Galanin levels in the homozygotes for the deletion were undetectable in all cases.

A comparison of levels of galanin expression between wild type, heterozygote and mutant mice in several body tissues is shown in Table 1.

Table 1

Genotype	Cortex	Hypothalamus	Anterior Pituitary	Stomach	Duodenum	Ileum
+/+	5.78±0.3 3	110.34±7.81	0.42±0.07	27.46±1.91	122.90±11.6 0	267.43 ±13.46
+/-	2.91±0.2 1	53.82±3.76	0.21±0.04	13.8±0.83	68.36±5.67	125.87 ±7.55
-/-	UD	UD	UD	UD	UD	UD

All values are mean galanin-LI pmol/g ± SEM, other than the female anterior pituitary which is expressed as pmol/gland ± SEM. UD=Undetectable

It will be seen that galanin was not detected in any of the tissues tested in the homozygous mutant mouse, and decreased by 50% in the heterozygous mutant mouse.

2. Although the mutant animals grow normally after weaning compared to their wild type litter mates (Fig 5) and achieve equal adult body weights, the same is not the case if the animals are weaned two days early. At P19 (i.e 19 days *post partum*) galanin would appear to be vital for the development of appetite for solids, if the animals are weaned at this point the mutants die within 48h. of starvation. Post mortem findings reveal a complete absence of food in the stomach or small bowel. Clearly this is a major finding

since very little is known about the normal regulation of appetite in the peri-weaning period. The mice of the invention are useful in studies on the expression of other neuropeptides known to regulate appetite (including leptin, neuropeptide Y, CCK, CRF and GLP-1).

3. The behavioural responses of intact adult mice to thermal and mechanical stimulation was tested. Responses to noxious thermal stimulus were measured using the Hargreaves paw withdrawal test (Hargreaves, K., Dubner, R., Brown, F., Flores, C. & Joris, J. *Pain* 32, 77-88 1988) and the sensitivity to mechanical stimulation was assessed with Von-Frey hairs (Woolf, C.J., Safieh Garabedian, B., Ma, Q.P., Crilly, P. & Winter, J. *Neuroscience* 62, 327-331 1994). No significant differences between homozygous mutants, heterozygotes and wild-type mice in either the withdrawal times in the hot plate test or sensitivity to mechanical stimulation (Fig. 6) were observed. Neuronal function does not appear to be compromised by the absence of galanin at least with respect to the sensory modalities tested.

4. Galanin is thought to play a role in the modulation of spinal cord transmission, particularly after nerve damage (axotomy) where its expression is upregulated during axonal regeneration. The response to axotomy is attenuated in the mutants (-/-) and autotomy fails to occur whilst self-mutilation in the wild type litter mates (+/+) is severe and occurs in almost all axotomised control animals (Fig. 7). The finding of hypo-algesia in the knockout mice is striking and unexpected. Previous data from Hökfelt's group in Sweden had suggested that galanin has a bimodal response on spinal cord transmission depending on the dose used.

5. The regenerative abilities of sensory axons in the sciatic nerve were directly measured by the pinch test (Danielsen, N., Kerns, J.M., Holmquist, B., Zhao, Q., Lundborg, G. & Kanje, M. *Brain Res.* 681, 105-108 1995). Following nerve crush, sensory axons regenerate into the distal nerve and can be stimulated by a subsequent nerve pinch, which elicits a reflex abdominal motor response. The foremost regenerating axons are located by pinching consecutive segments of the nerve in a distal to proximal direction until a reflex is observed and the distance from the nerve crush can be calculated.

Regeneration showed a statistically significant reduction of 30-40% in homozygotes compared to wild type mice at 2, 4 and 6 days after nerve crush (Fig. 8). Regeneration was intermediate in heterozygous mice but was still significantly different from wild type animals.

To test whether the reduced rate of regeneration in galanin-deficient mice affects functional recovery after a crush injury, we tested a behavioural correlate of regeneration using the toe spreading index (Hoogeveen, J.F., Van Der Kracht, A.H., Wondergem, J., Gonzalez Gonzalez, D. & Haveman, J. *Neurotoxicology*. 14, 1-7 1993). Rodents spread the toes on their hind feet upon contact with a solid surface, a response which requires sensory innervation. Toe spreading is, therefore, lost after axotomy until sensory axon re-innervation occurs. The toe spreading distance was measured for 6 weeks after unilateral right sciatic nerve crush and compared to the intact contralateral (left) foot. Whilst toe spreading in wild-type mice returned to normal within 3 weeks of sciatic nerve crush, functional regeneration was still incomplete at six weeks in the mutant mice (Fig 9).

6. The decreased regeneration and autotomy in the galanin-deficient mice might be related to the death of neurons following axotomy, especially those neurons which would normally express galanin after injury. To test whether galanin is essential for the survival of neurons during development, we studied the distribution of galaninerbic neurons in wild type and mutant mice. Since we were unable to visualise the galaninerbic neurons in the mutant animals at the protein level we studied expression of the mRNA using a riboprobe specific for exon six (marked B, see Figure 3). In order to confirm the survival of other populations of galanin expressing neurons, the exon 6-specific riboprobe was used to visualise galaninerbic neurons in the hippocampus and the paraventricular nucleus of the hypothalamus of adult wild-type and mutant mice (Fig 10). No differences in expression were observed between the groups suggesting that neuronal development are normal in these animals and not galanin dependent.

We went on to use the exon 6-specific riboprobe to study the distribution of galaninerbic neurons in the DRG two weeks after sciatic nerve axotomy. A marked up-regulation in the

levels and number of cells expressing galanin was observed in the DRG neurons of wild type mice (Fig 10). However, there was no expression in the homozygous galanin-deficient mice, suggesting that galanin is required for these cells to survive axotomy.

These results relating to regeneration and cell survival are particularly significant in that the results indicate that galanin gene is the first gene to affect regeneration of the peripheral nervous system.

Accordingly, the invention contemplates the use of a galanin agonist in the treatment of peripheral sensory neuropathy resulting, for example, from diabetes mellitus or trauma (such as that caused by traffic accidents).

7. Homozygote mutants enter puberty at the same time as their litter mate controls, pregnancy and resulting litter size appeared unaffected. Mutant females, however, are unable to lactate and all pups died of dehydration/starvation unless fostered by wild type mothers. Pituitary prolactin content and secretion is reduced some five fold in pregnant homozygotes (-/-) compared to pregnant wild type (+/+) controls killed 4 days after birth (Fig. 11) but is only 80% of normal in randomly cycling female homozygote mice.

The addition of exogenous oestradiol (0.5µg of 17 β-oestradiol given subcutaneously as a suspension in linseed oil) to rodents has a strong mitogenic effect on pituitary cell number and markedly increases pituitary prolactin content (Fig. 11).

These effects are abolished in the knockout mice, confirming that galanin is crucial to lactotroph growth and to prolactin secretion in the hyperoestrogenised state. These findings coupled with previous data that galanin induces growth of the lactotroph, combine to substantiate the hypothesis that an activating mutation in the pituitary galanin receptor may be responsible for the formation of prolactinomas (prolactin secreting pituitary tumours).

Anterior pituitary content for three other hormones was assessed. No differences were found in the content of TSH, GH and LH (figs 12-14) in mutant versus wild type mice.

It would be expected that the mutant mouse of the invention would have high insulin and low plasma glucose. Thus galanin antagonists might be of use in treatment of diabetes mellitus.

Galanin may inhibit hypothalamic somatostatin release thus stimulating growth hormone. One would expect the mutant mice to have high levels of somatostatin, low GH and to be small. Thus galanin might be a treatment for idiopathic small stature.

Such changes caused by the mutations to the mouse of the invention as disclosed above have implications for possible treatments of a number of human conditions/diseases using either galanin agonists or antagonists. Such diseases may include:- anorexia, obesity, painful neuropathies, pituitary prolactin secreting tumours, Alzheimer's dementia and diabetes.

8. Galanin has been implicated in the aetiology of Alzheimer's disease. Hippocampal galanin expression is increased in cholinergic neurones as acetylcholine and choline acetyl transferase (ChAT) levels fall. Administration of galanin decreases learning behaviour in a number of mouse models, the converse is also true when galanin antagonists are infused. We measured long term potentiation (LTP) in wild type and mutant mice. LTP is an electrophysiological test where specific nerves in the hippocampus are stimulated by an electric shock: Davies CH, Collingridge GL. *J. Physiol. Lond.* 1996;**496**: 451-470; Davies CH, Starkey SJ, Pozza MF, Collingridge GL. *GABA Nature* 1991;**349**:609-611. This procedure is done *in-vitro* using brain slices from recently killed animals. Results show that LTP is decreased by 50% in the stratum oriens at the 80 minute time point in the mutants compared to wild-type mice (Fig 16 A vs C). In contrast no difference was found in LTP measured in the stratum radiatum. Galanin is found at high levels in the stratum oriens but NOT in the stratum radiatum. Our data, thus far, demonstrates a decrease in LTP in the mutants implying a decrease in memory and cognition - tests to assess these function are being conducted. These data show that a galanin agonist is useful in the treatment of Alzheimer's disease and associated memory loss with an enhancement in memory and cognition.

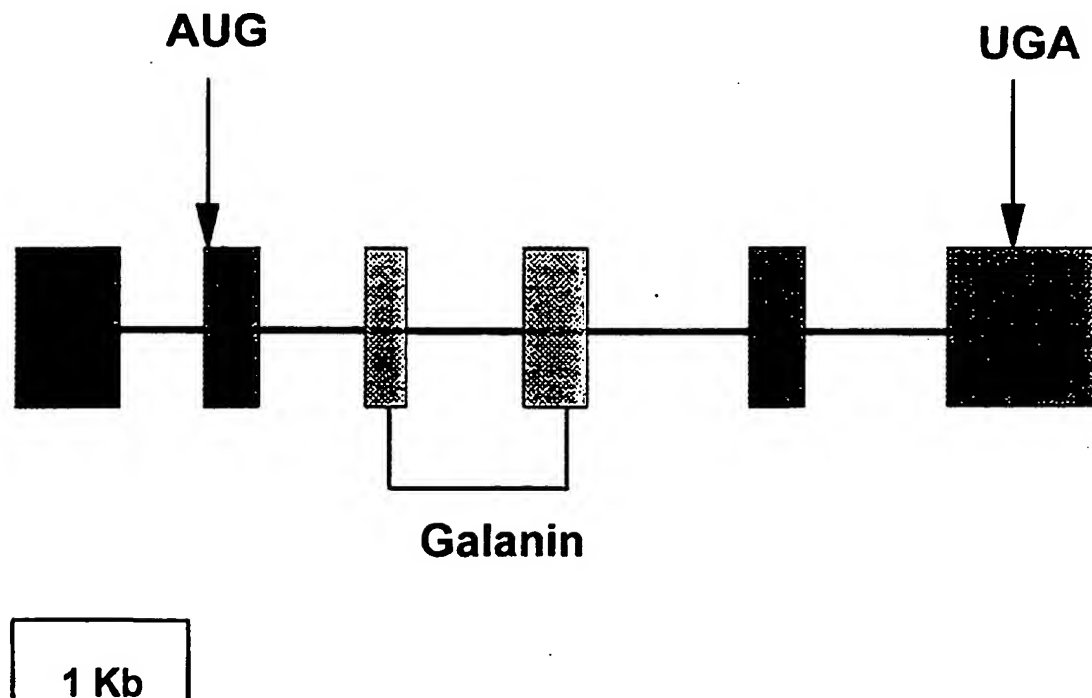
1. The use of a galanin agonist in the preparation of a medicament for the treatment of nerve damage.
2. A method of treating nerve damage in a mammal comprising administering a galanin agonist to that mammal.
3. A method of treating Alzheimer's disease and related diseases and conditions comprising administering a galanin agonist to a subject.
4. The use of a galanin agonist in the preparation of a medicament for the treatment of Alzheimer's disease and related diseases and conditions.
5. A method of improving memory, enhancing memory functions and improving cognitive function, the method comprising administering a galanin agonist to a subject.
6. The use of a galanin agonist in the preparation of a medicament for improving memory and other cognitive functions.
7. A lactation suppression composition comprising a galanin antagonist.
8. The use of a galanin antagonist in the preparation of a medicament for the suppression of lactation.
9. A method of suppressing lactation in a mammal, the method comprising administering a galanin to that mammal.
10. A composition comprising a galanin antagonist for the treatment of prolactinoma in a mammal.
11. The use of a galanin antagonist in the preparation of a medicament for the treatment of prolactinoma.

12. A method of treating prolactinoma in a mammal suffering from prolactinoma, the method comprising administering a galanin antagonist to that mammal.
13. An appetite suppressant composition comprising a galanin antagonist.
14. The use of a galanin antagonist in the preparation of a medicament for the treatment of appetite, and appetite related disorders.
15. A method of suppressing appetite in a mammal, the method comprising administering a galanin antagonist to that mammal.
16. An analgesic composition comprising a galanin antagonist.
17. The use of a galanin antagonist in the preparation of a medicament for the treatment of pain.
18. A method of suppressing pain in a mammal, the method comprising administering a galanin antagonist to that mammal.
19. The use of a galanin antagonist in the preparation of a medicament for the treatment of painful neuropathy.
20. An anaesthetic composition comprising a galanin antagonist.
21. The use of a galanin antagonist in the preparation of an anaesthetic composition.
22. A method of anaesthetising a mammal, the method comprising administering a galanin antagonist to that mammal.
23. A transgenic or other genetically modified mammal which lacks a functional galanin gene.

24. A mammal according to claim 23 in which the galanin gene has been inactivated.
25. A mammal according to claim 23 or 24 in which the galanin gene has been inactivated by at least partial deletion.
26. A mammal according to claim 25 in which the portion of the galanin gene between the Bam HI and Bgl2 restriction sites asterisked in Fig. 3 has been deleted.
27. A mammal according to claim 23, 24, 25 or 26 which is a rodent.
28. A rodent according to claim 27 which is a mouse.
29. Tissue, cells and cell lines derived from a mammal, rodent or mouse according to any preceding claim.
30. Tissue, cells or cell lines according to claim 29 which are cells from pancreas, pituitary, cortex, dorsal root ganglia or are derived from such cells.
31. The use of a mammal, rodent or mouse according to any one of claims 23 to 28 or tissue cells and cell lines according to claim 29 or 30 in an assay to determine a biological effect of galanin.
32. The use according to claim 31 in which the biological effect is selected from diabetes and insulin secretion, appetite, growth hormone effects, lactation, prolactin over secretion, pain sensitivity, memory, behaviour, sexual reproduction and fertility.

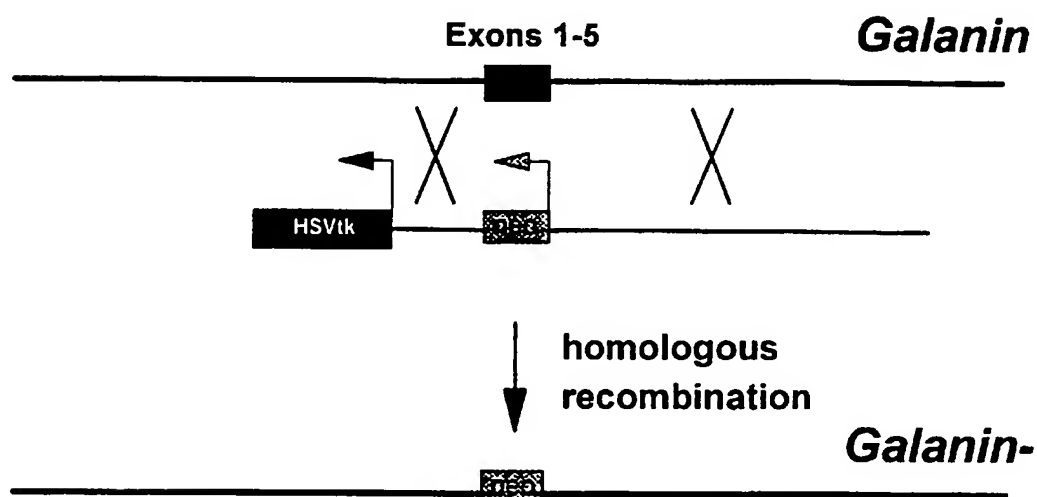
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Fig1
GENOMIC
STRUCTURE mGAL



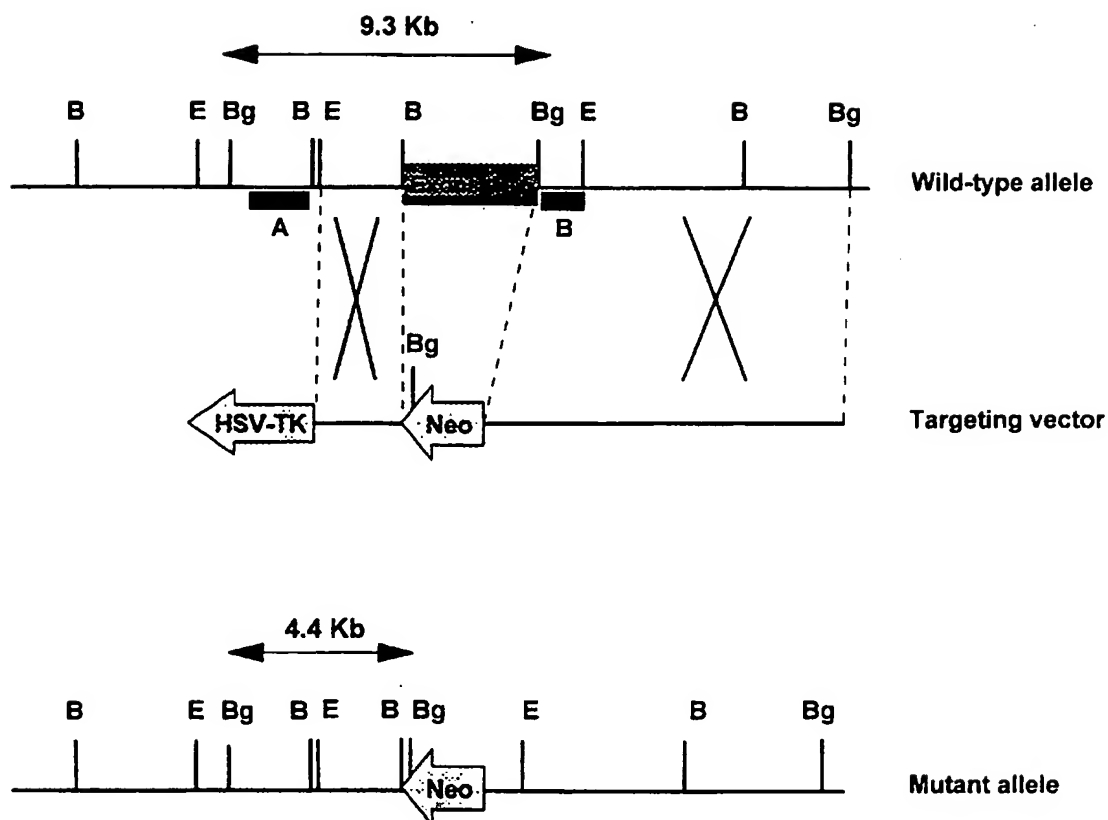
2/16

TARGETING VECTOR



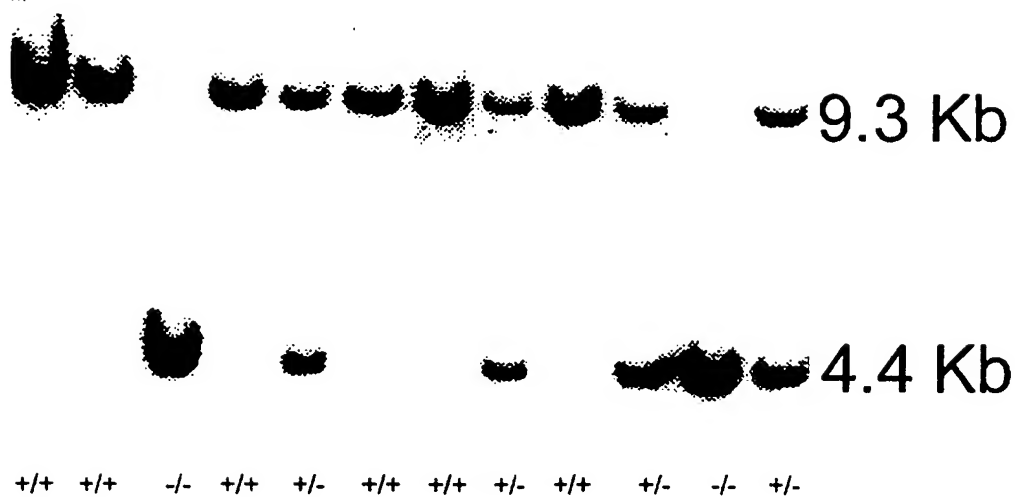
3/16

Figure 3



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Figure 4

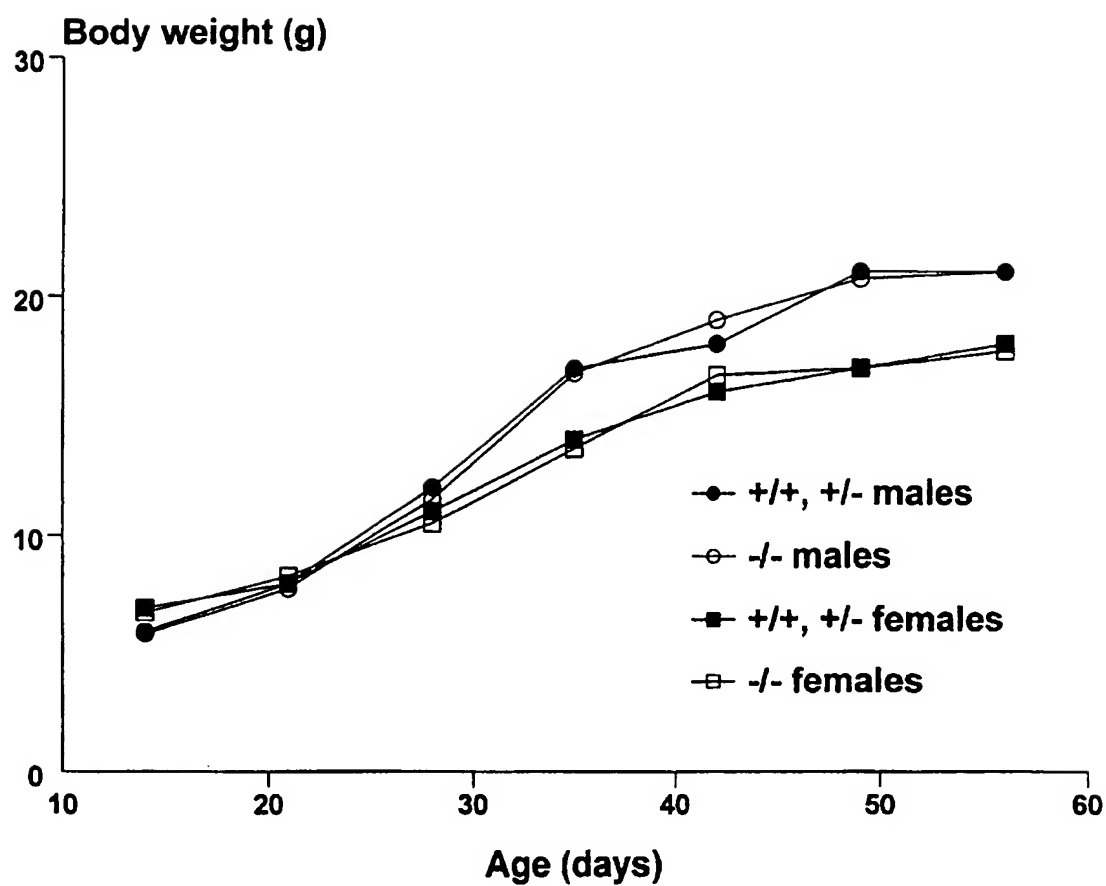
*Bgl*II

PCR



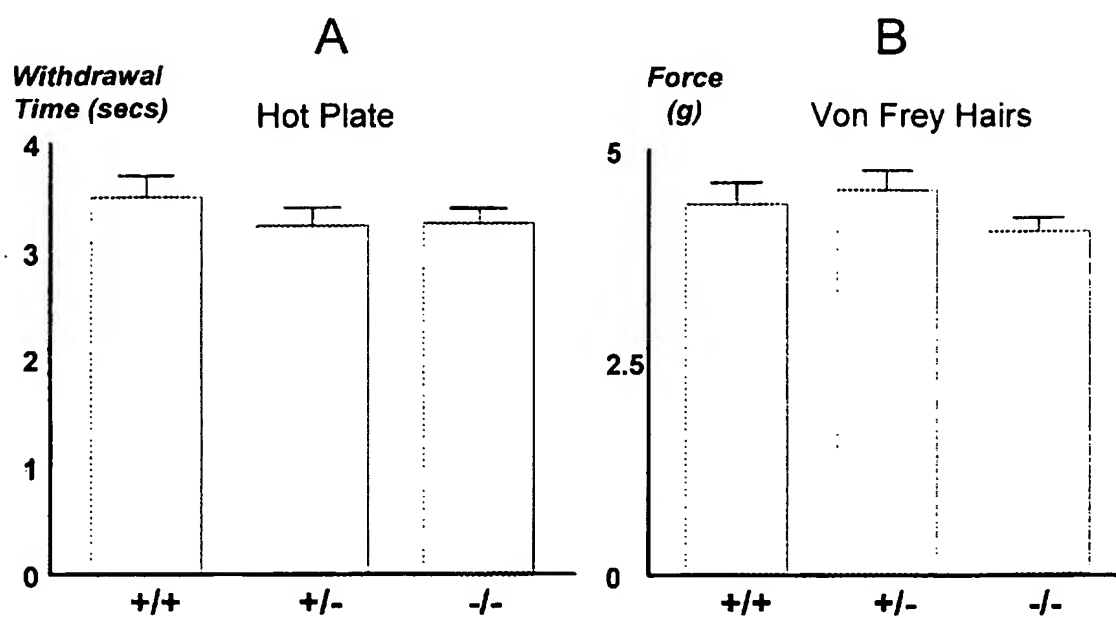
5/16

Figure 5



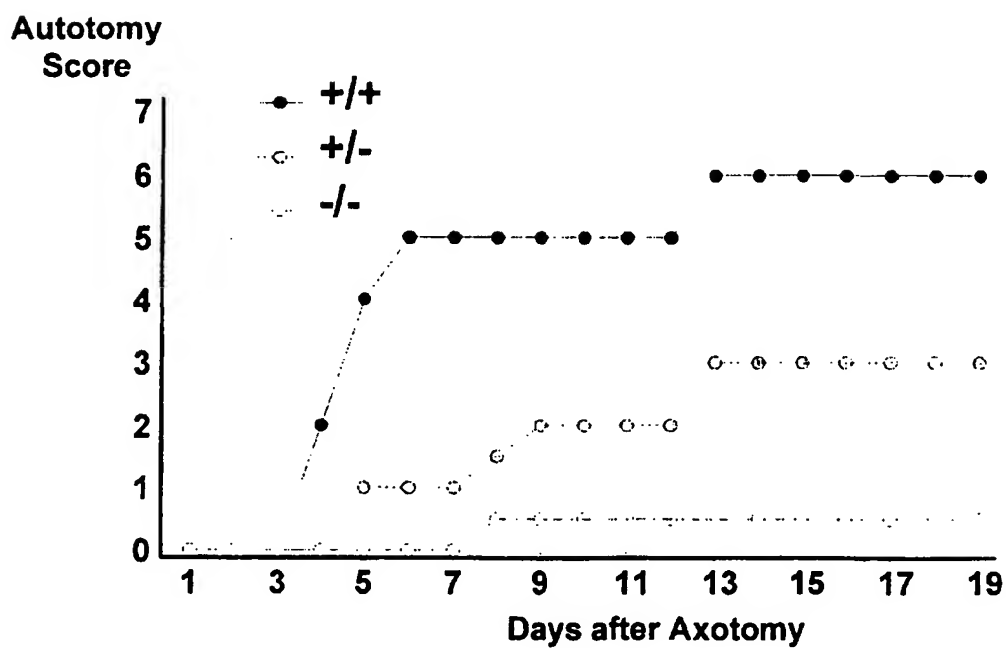
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Figure 6
Effect of Galanin Disruption on
Heat and Mechanical Sensitivity



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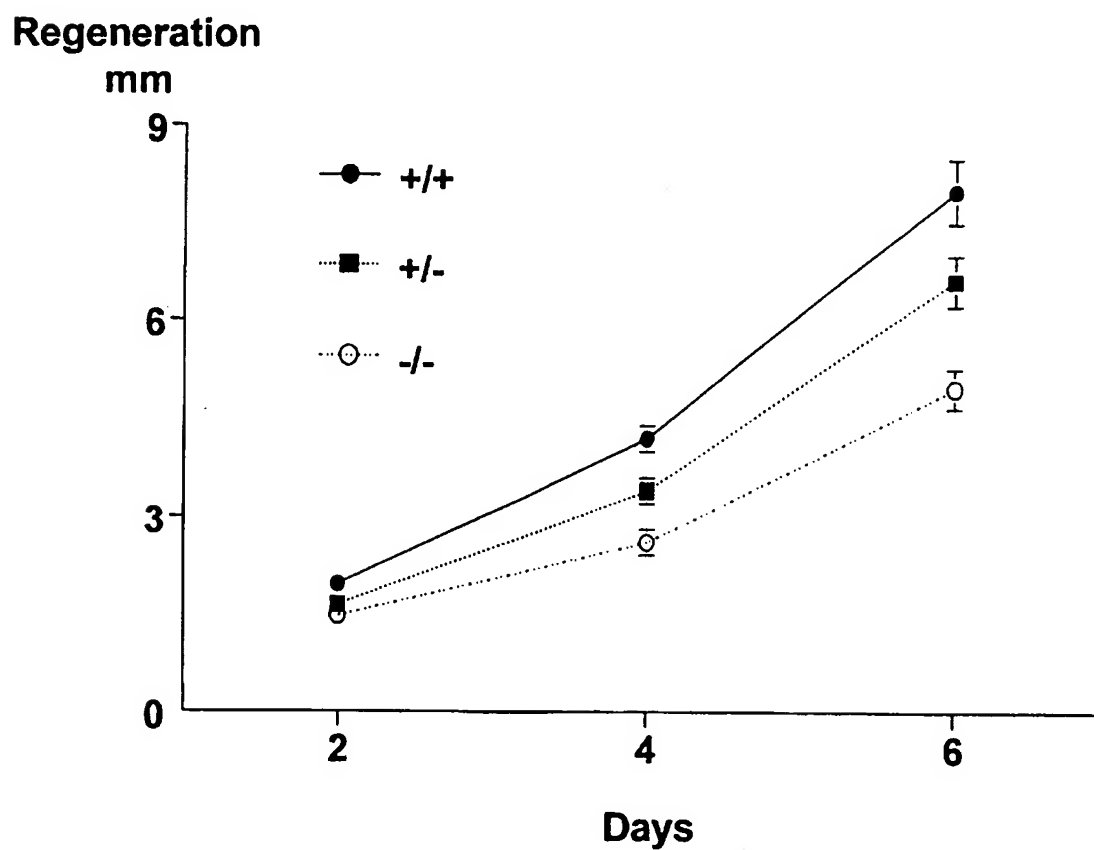
Figure 7
Effect of Galanin Disruption on Chronic Neuropathic Pain



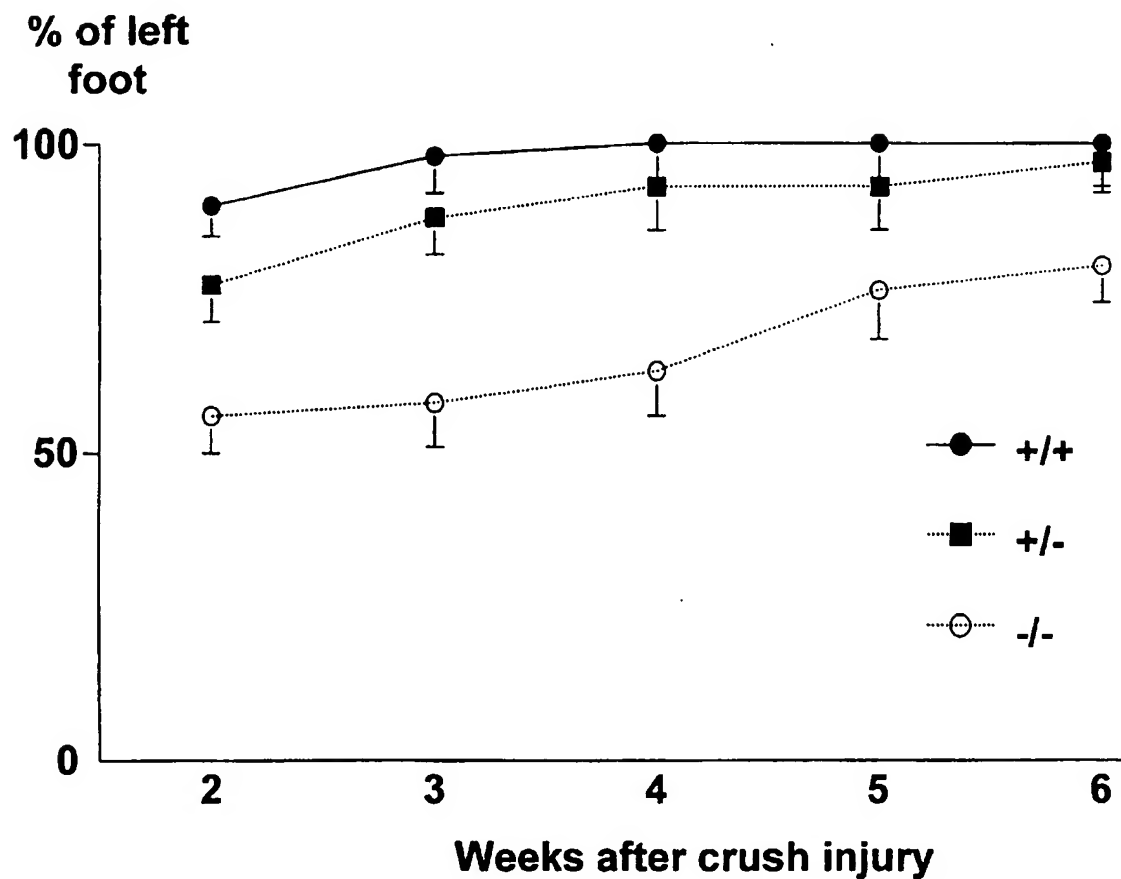
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Figure 8

Regenerative Rates After a Crush Injury to the Right Sciatic Nerve

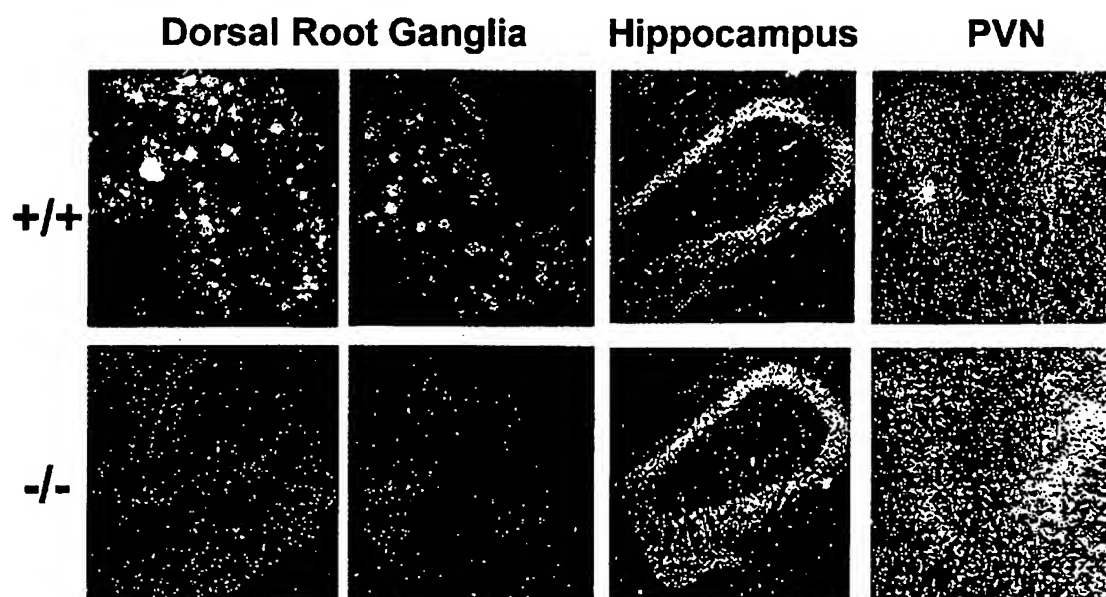


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Figure 9**Toe spreading index after a crush injury to the right sciatic nerve**

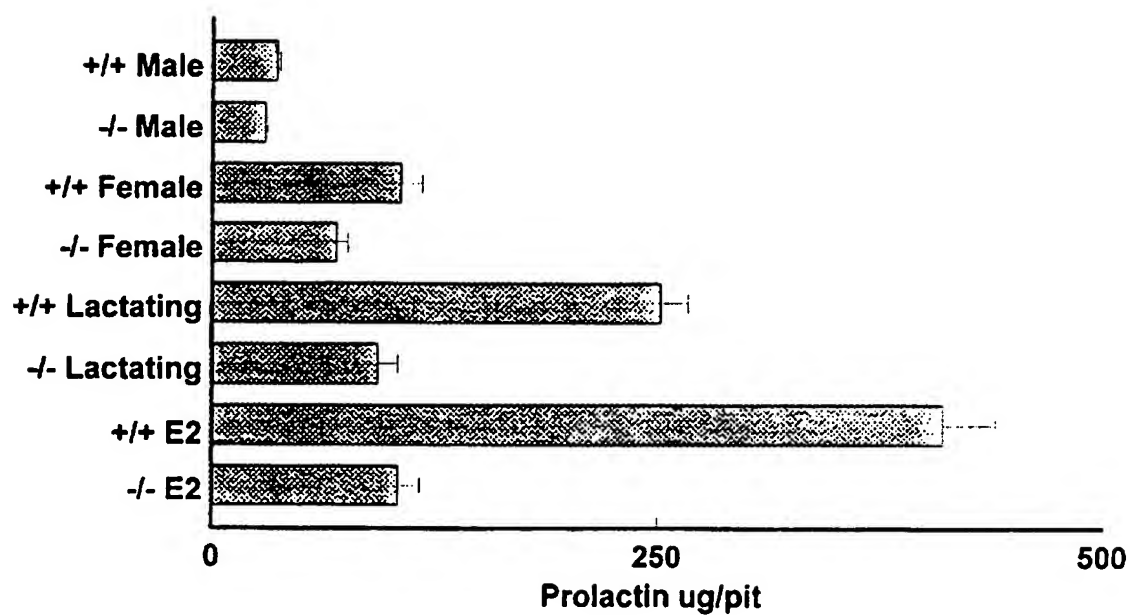
10/16

Figure 10
In-situ hybridization using Exon 6 riboprobe



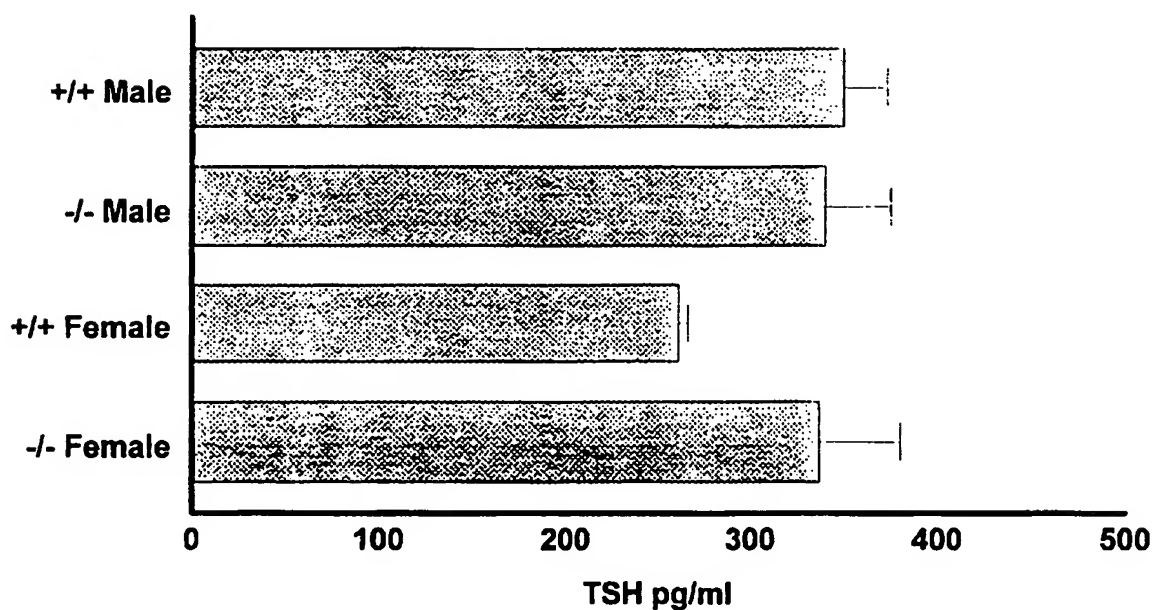
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Figure 11
ANTERIOR PITUITARY CONTENT



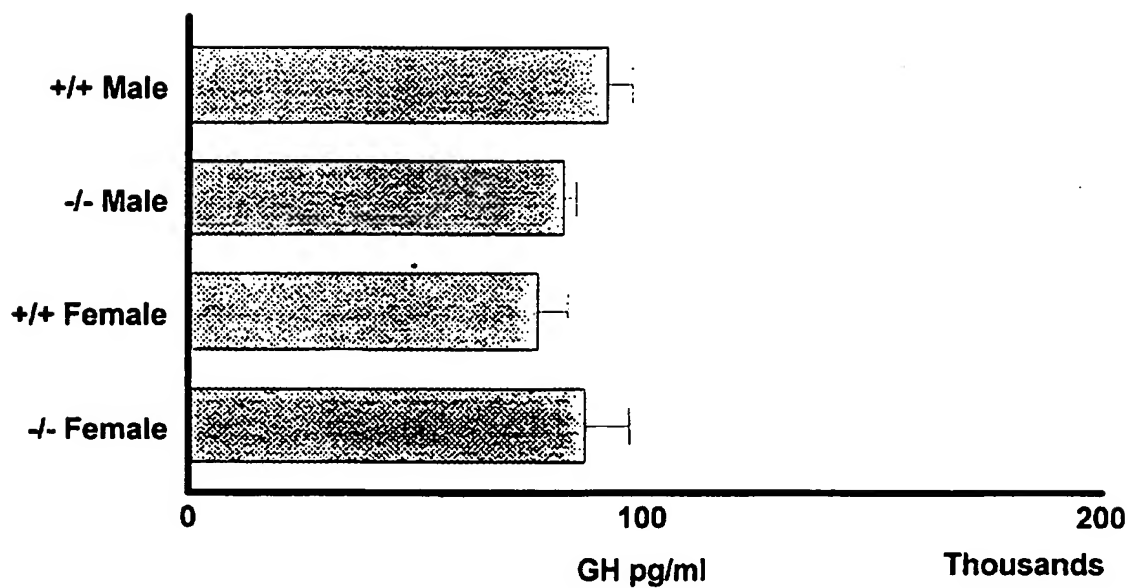
12/16

Figure 12
ANTERIOR PITUITARY CONTENT



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Figure 13 ANTERIOR PITUITARY CONTENT



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Figure 14

ANTERIOR PITUITARY CONTENT

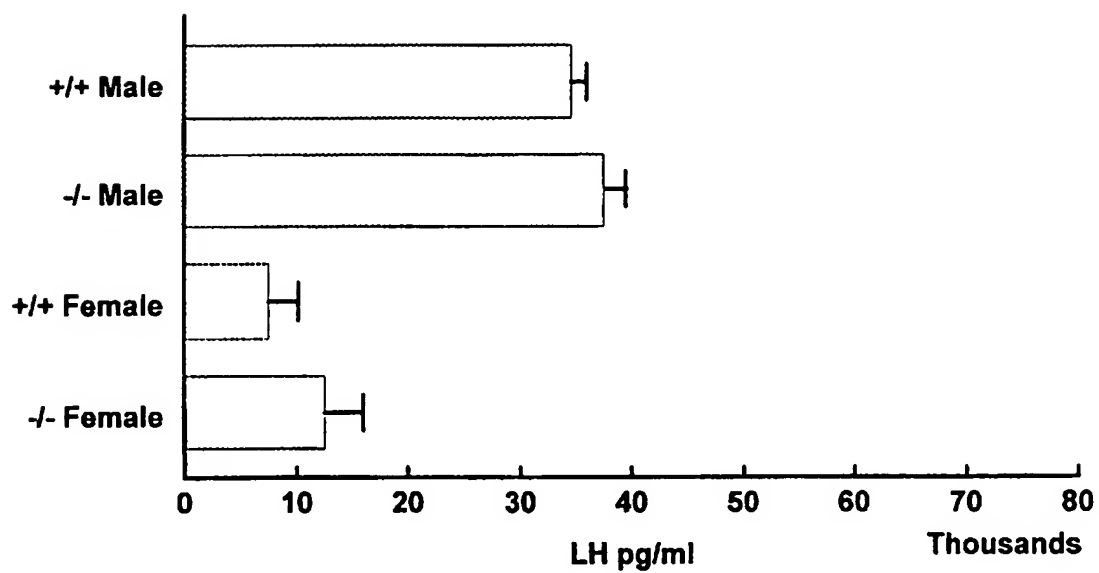
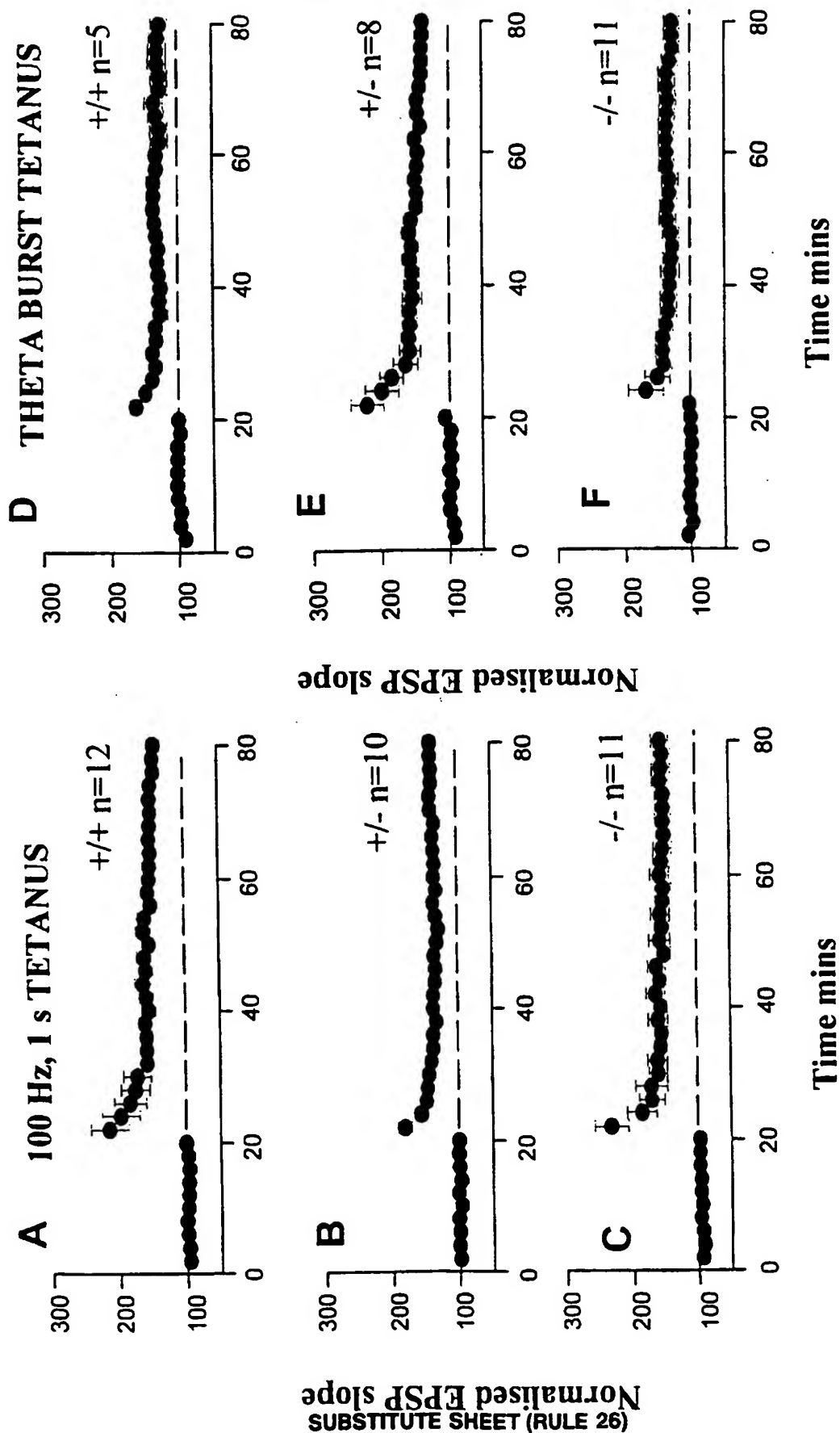


FIGURE 15

LONG-TERM POTENTIATION IN STRATUM RADIATUM

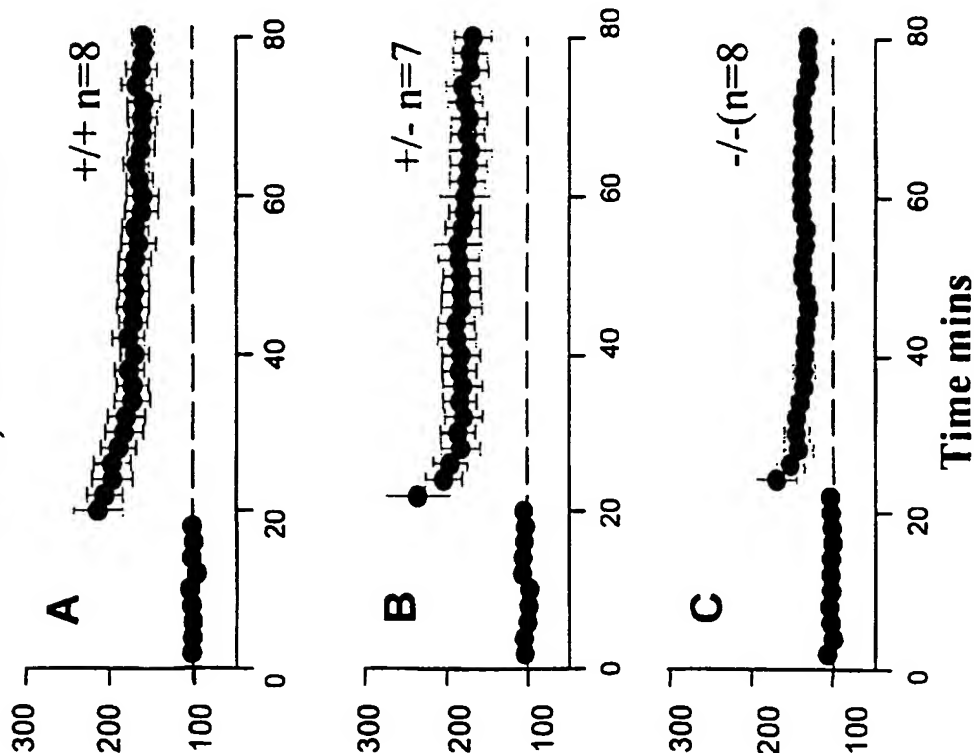


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FIGURE 16

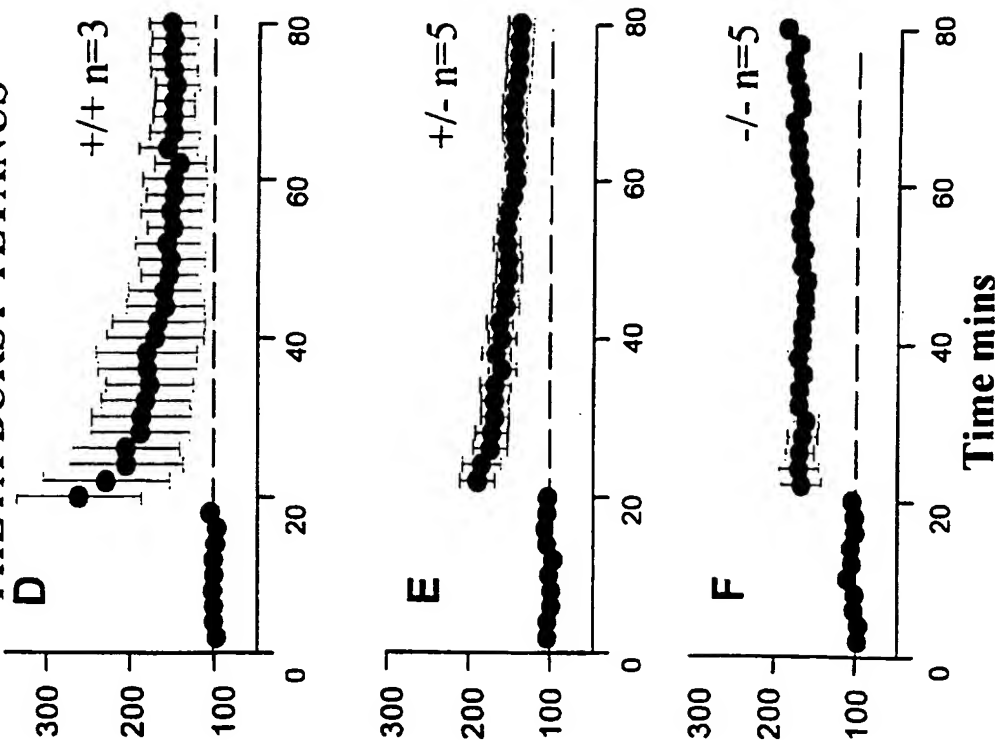
LONG-TERM POTENTIATION IN STRATUM ORIENS

100 Hz, 1 s TETANUS



Normalised EPSP slope

THETA BURST TETANUS



Time mins

Time mins

Normalised EPSP slope
SUBSTITUTE SHEET (RULE 26)

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 97/01991

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A01K67/027 A61K38/22 C12N15/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K A01K C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 92 20709 A (AKTIEBOLAGET ASTRA) 26 November 1992 see the whole document ---	1-22
X	WO 92 12997 A (THE GENERAL HOSPITAL CORPORATION) 6 August 1992 see the whole document ---	1-22
X	DATABASE WPI Section Ch, Week 9429 Derwent Publications Ltd., London, GB; Class B04, AN 94-238764 XP002045652 & JP 06 172 387 A (AIBAITSU KK) , 21 June 1994 see abstract --- -/--	1-4

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

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- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
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- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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- *S* document member of the same patent family

Date of the actual completion of the international search

6 November 1997

Date of mailing of the international search report

17. 12. 97

Name and mailing address of the ISA

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Fax: (+31-70) 340-3016

Authorized officer

Moreau, J

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 97/01991

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>BARTFAI T ET AL: "GALANIN AND GALANIN ANTAGONISTS MOLECULAR AND BIOCHEMICAL PERSPECTIVES." TRENDS PHARMACOL SCI 13 (8). 1992. 312-317, XP002045935 see the whole document ---</p>	1-4, 16-19
X	<p>UKAI M ET AL: "Effects of galanin on passive avoidance response, elevated plus-maze learning, and spontaneous alternation performance in mice." PEPTIDES (TARRYTOWN) 16 (7). 1995. 1283-1286, XP002045936 see the whole document ---</p>	5,6
X	<p>WO 92 15681 A (GARVAN INSTITUTE OF MEDICAL RESEARCH) 17 September 1992 cited in the application see the whole document ---</p>	1-22
X	<p>WO 92 15015 A (ZYMOTENETIC, INC) 3 September 1992 cited in the application see the whole document ---</p>	1-22
X	<p>WYNICK D ET AL: "GALANIN REGULATES BASAL AND OESTROGEN-STIMULATED LACTOTROPH FUNCTION." NATURE (LOND) 364 (6437). 1993. 529-532, XP002045651 see the whole document ---</p>	7-12
E	<p>WO 97 26853 A (SYNAPTIC PHARMACEUTICAL CORPORATION) 31 July 1997 see the whole document -----</p>	1-32

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 97/01991

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/GB 97/01991

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Remark : Although claims 2,3,5,9,12,15,18,22 are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter. Appl. No.

PCT/GB 97/01991

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